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13. ABSTRACT (Maximum 200 Words)

The purpose of this research is to investigate whether eating brown seaweed (*Undaria pinnatifida*) can influence breast cancer risk. Brown seaweeds are popular in Japan, where the incidence of breast cancer is about 1/6 the rate of that reported for American women. In several animal studies of diet and cancer, adding seaweed to the normal diet resulted in longer healthy lives. In particular, we will examine cell-cell adhesion and gene expression associated with the consumption of dietary seaweeds by women who are healthy and women who have breast cancer. We will use commercially available seaweed. These seaweeds are commonly found in health food stores.

To date, progress has been limited by the lack of approval from HSRRB (Proposal Log Number BC972552, HSRRB Log Number A-8050), with modifications. Modifications were submitted August 26, 2003. Final approval has not yet been granted. However, when this grant was first awarded in 1999 to the University of Massachusetts, we did a preliminary study to assess potential toxicity of dietary seaweed. The first paper on iodine content in commercially available seaweeds is now in press, and the second paper on bioavailability of seaweed iodine has been submitted for publication.

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INTRODUCTION

Breast cancer is the second leading cause of cancer among American women. Survival rates at 5 years average 87%, decreasing to 77% at 10 years, 63% at 15 years, and 52% at 20 years(*1*). Although current use of tamoxifen and letrozole may have a significant impact on long term survival in the future, an estimated 39,800 American women will die of breast cancer in 2003 (*1*). There is an urgent need for new treatments for metastatic breast cancer and chemoprevention that can be used to prevent breast cancer recurrence.

Epidemiologic studies comparing breast cancer rates among Japanese women in Japan and American women in the US are supportive that dietary factors could be critical to understanding breast cancer rates. In vitro work using seaweed extracts have shown high antitumor activity. In vivo work using rats and mice have demonstrated that seaweed, both as part of a regular diet, as an extract in drinking water, and as extracts which were injected into tumor bearing rats, have all confirmed that something in seaweed inhibits cancer formation and can cause tumor remission/tumor rejection in tumor bearing animals.

Fucoidans are sulfated sugar molecules only found in brown seaweeds and have been widely studied for their ability to inhibit cell-cell adhesion. Metastatic breast cancer cells attach to potential sites of metastases (primarily lung, liver, and bone) via binding of the CXCR4 cell protein to stromal derived factor – 1 (SDF1). In other studies we have found that CXCR4 on lymphocytes in healthy individuals is blocked after consuming dietary seaweed, possibly by increased circulating levels of SDF1.

In this study we propose dietary algae (*Undaria pinnatifida)* as a safe inexpensive supplement that may modulate the motility, homing, and proliferation of metastatic breast cancer cells. Metastatic breast cancer cells overexpress CXCR4 chemokine receptors (*2*). Neutralization of CXCR4 interactions with its ligand stromal derived factor 1 (SDF1 or CXCL12) has been shown to inhibit metastases (*2*). CXCL12 are most abundantly present at the sites of the most common breast cancer metastases in lung, liver, and bone marrow (*3*). Injection of the algae sulfated polysaccharide fucoidan has been shown to cause a significant dislodgement of CXCL12 from its normal adhesion to endothelial cells (*4, 5*), and in a pilot study dietary algae decreased CXCR4 expression (possibly by CXCL12 binding) by 47% as measured by flow cytometry. We propose that the free CXCL12 will act as a decoy-binding site for metastatic breast cancer cells. Since there is also a CXCR4 binding site on antigen presenting dendritic cells, an increase in CXCL12 – bound breast cancer cells may activate helper T cells to specifically target these CXCL12-bound cancer cells. A second mechanism associated with breast cancer metastases is an increase in CD24 expression, the ligand for P-selectin (*6*). In a pilot study of dietary algae we demonstrated a significant increase in free P-selectin (CD62P), suggesting that CD24 expression would be down regulated, and could be a second pathway that algae uses to decrease breast cancer metastases. Our endpoints will be measurement of CXCR4, CXCL12, CD62P, and CD24. In addition, we will investigate the effect of dietary algae on circulating breast cancer cells.

This will be a randomized double-blinded crossover study that includes 15 women (5 healthy women, 5 who have been treated for early breast cancer but are currently disease free, and 5 women who have been treated for Stage III breast cancer but are currently not receiving treatment and are considered at high risk for recurrence). The 15 women would be randomized to seaweed or placebo first, have their blood drawn at baseline, take 5 g/d (10 capsules) of *Undaria pinnatifida* or placebo for 2 weeks, have their blood drawn again, and take placebo for two week washout period, have their blood drawn and take 10 capsules of placebo or *Undaria* (which ever not taken the first time) for 2 weeks, and have their blood drawn again. Altogether we would have 4 time points. We will use flow cytometry to measure changes in the relative fluorescence of CXCR4, CD24, and bound platelets (CD62P). In addition we will use a DNA microarray approach to identifying patterns of gene expression encoding for cell adhesion and innate and adaptive immune response. This approach will also allow us to investigate the interrelationships between gene regulation that may be important in breast cancer surveillance and tumor responses.

The DNA microarray analysis would be done only after 2 weeks on placebo and 2 weeks on *Undaria*. In addition to the blood needed for the DNA microarray, the blood samples from the 4 visits would be collected, centrifuged, and 1 ml aliquots of plasma would be stored at –80. These samples would be used in ELISA assays to quantify changes in SDF1.

BODY

This research project was begun in 1999, at the University of Massachusetts. However, only the initial work on seaweed toxicity was completed before the PI moved to the University of South Carolina. This coincided with the necessity of obtaining Army IRB approval, and although numerous renditions of the grant have now been made, and tentative Army IRB approval was given in August 2003, official permission to begin the study has not been obtained. All requested documents were mailed to the Army on August 26, 2003.

The original work begun at the University of Massachusetts has now been completed. We first assessed the potential toxicity of dietary seaweeds for use in our research by collecting and analyzing commercially available dietary seaweeds for their iodine content. The paper reporting our findings has now been accepted by *Thyroid*. Based on our work, we identified Undaria pinnatifida, Alaria esculenta, and Sargassum muticum as having safe levels of iodine. In a second study done at the University of Massachusetts on the bioavailability of seaweed iodine has now been submitted to the *American Journal of Clinical Nutrition*. Our conclusions were that although 5 grams/day of seaweed, the average daily consumption in Japan, was associated with a statistically significant increase in thyroid stimulating hormone, the increase was small and not biologically important. All clinical values remained within normal limits. This means that our next intervention will be done with a clinically proven safe level of iodine-containing seaweed. The two manuscripts are included in the appendix.

KEY RESEARCH ACCOMPLISHMENTS

Evaluation of iodine content in commercially available dietary seaweeds
Evaluation of bioavailability of seaweed iodine

REPORTABLE OUTCOMES:

Manuscripts:

Jane Teas, Lewis E. Braverman, Mindy S Kurzer, Sam Pino, Thomas G. Hurley, James R. Hebert. Seaweed and Soy: Companion Foods in Asian Cuisine and Their Effects on Thyroid Function in American Women. ***Thyroid***. In Press

Jane Teas, Sam Pino, Alan Critchley, Lewis E. Braverman.Variability of Iodine Content in Common Commercially Available Edible Seaweeds. Submitted to ***American Journal of Clinical Nutrition***.

Poster presentations

Effect of Seaweed Ingestion on Thyroid Function in Postmenopausal Women (P3-675). **(Jane Teas**, Sam Pino, Thomas G. Hurley, Alan Critchley, Lewis E. Braverman). **Endocrinology Society** Annual Meeting, Philadelphia, PN June 2003.

Funding applied for and awarded

Dietary Algae as a Modulator of Breast Cancer Metastases: An exploratory Grant to Document Proof of Principle **(Principal Investigator: Jane Teas).** Cancer Prevention and Cancer Control (Department of Defense Award to encourage collaboration between the Medical University of South Carolina and the University of South Carolina). Awarded December 2003.

Dietary Algae and Breast Cancer. University of South Carolina preliminary grant to be used in application for NIH funding of a Cancer Complementary and Alternative Medicine Center. **(Principal Investigator: Jane Teas**). Awarded May 2004.

CONCLUSIONS

My major conclusion is that my grant appears to have been somehow lost in the Army IRB process. Although we have data on which seaweed to use in our study, it would be nice to begin our study. The requested documents have been submitted to the Human Subjects Protection Specialist, and I await permission to begin my clinical study.

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APPENDICES

1. Jane Teas, Sam Pino, Thomas G. Hurley, Lewis E. Braverman. Effect of seaweed ingestion on thyroid function in postmenopausal women. Abstract P3-675 Presented at the **Endocrinology Society** Annual Meeting, Philadelphia, PN June 2003.

2. Jane Teas, Sam Pino, Alan Critchley, Lewis E. Braverman.Variability of Iodine Content in Common Commercially Available Edible Seaweeds. ***Thyroid***. In Press

3. Jane Teas, Lewis E. Braverman , Mindy S Kurzer, Sam Pino, Thomas G. Hurley, James R. Hebert. Seaweed and Soy: Companion Foods in Asian Cuisine and Their Effects on Thyroid Function in American Women. Submitted to ***American Journal of Clinical Nutrition***.

P3-675

APPENDIX 1

Effect of Seaweed Ingestion on Thyroid Function in Postmenopausal Women

Jane Teas, Sam Pino, Thomas G. Hurley, Lewis E. Braverman

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Abstract

We have previously reported that there was a significant ($p < 0.007$) delay in the development of DMBA-induced mammary tumors in seaweed treated rats compared to control rats. Breast cancer rates are significantly higher in the US than in Asia where seaweed is a staple in the Asian diet. Prior to determining whether seaweed ingestion could possibly affect the occurrence of breast cancer in US women, a study was carried out to determine the effect of the ingestion of iodine (I) containing seaweed in healthy, postmenopausal American women. Commercially available samples of 12 different kinds of edible seaweeds were analyzed for iodine content and found to range from 16 ug/g to 8165 ug/g. We recruited twenty-five women (mean age 58 yr) to a double-blinded randomized crossover study. Five gm/d of encapsulated seaweed (*Alaria esculenta*) containing 550 ug I or placebo capsules were given to the women as follows: 13 women seaweed (A) and 12 women placebo (B) for 6 weeks, a 3 week washout period, followed by group A placebo and group B seaweed. Thus, 25 women received seaweed or placebo for 6 weeks. Urine I and serum T4, FTI, TT3 and TSH concentrations were compared at the termination of each 6 week course of treatment. Urinary I concentrations increased significantly to 568 ± 32 ug/day (mean ± SE) in the seaweed-treated women compared to 266 ± 32 ug/day on placebo ($p<0.0001$). There were no significant changes in serum T4, FTI, and TT3 concentrations during seaweed or placebo ingestion. However, seaweed ingestion induced a significant rise in the serum TSH concentrations due to the excess I (1.69 ± 0.22 on seaweed vs 2.19 ± 0.22 µU/ml on placebo, $p < .0001$). Conclusion: Ingestion of seaweed containing I induced small but significant increases in serum TSH concentrations in healthy, postmenopausal women. Whether further abnormalities of thyroid function would occur during more prolonged ingestion of I rich nutrients remains unclear.

Study Design

Randomized Double Blinded Crossover

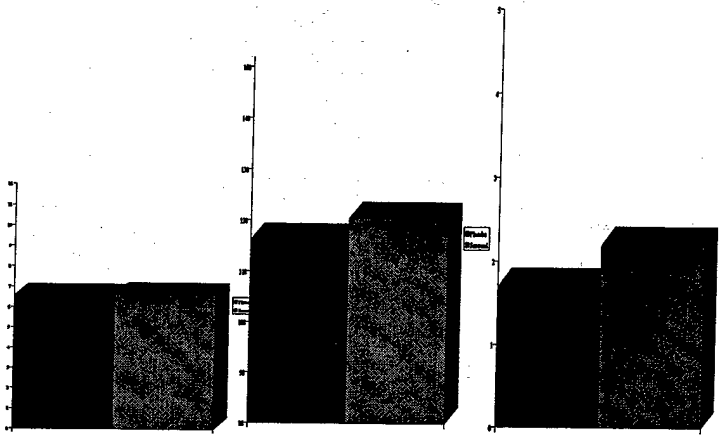
Seaweed 6 wks → Washout 3 wks → Placebo 6 wks

Placebo 6 wks → Washout 3 wks → Seaweed 6 wks

Subject demographics

	Breast Cancer n=10	Disease free n=15
Age (yr) ± SD	58.4 ± 6.1	58.1 ± 8.5
Age at Menopause (yr)	48.8 ± 3.5	50.5 ± 2.2
BMI ± SD	27.2 ± 6.7	26.2 ± 4.4
Employed	80%	67%
> High School	80%	73%

Serum Thyroid Hormones



T4 ug/dl ns

T3 ng/dl ns

TSH uU/ml P = 0.0001

Seaweed Iodine

Seaweed Iodine By Genus

Iodine Content of Common commercially Available Seaweeds

Name	Location	Form	N	µg/g	±SD
Alaria (Alaria esculenta)					
	Maine	whole	7	110	30
	Maine	whole	5	431	104
Knotted wrack (Ascophyllum nodosum)					
	Maine	whole	3	646	392
Arame (Eisenia bicyclis)					
	Japan	whole	3	586	56
Hijiki (Hizikia fusiforme)					
	Japan	whole	6	629	153
Kelp (Laminaria)					
L. longicurus					
	Maine	whole	3	746	26
			6	1882	520
L. saccharina					
	Canada	capsule	5	1259	209
L. digitata					
	Maine	whole	6	1997	563
	Iceland	granules	6	8165	373
L. angustata					
	Japan	powdered	4	2353	65
Dulse (Palmaria palmata)					
	Maine	whole	3	72	23
Nori (Porphyra tenera)					
	Japan	sheet	3	16	2
Sea Palm (Postelsia palmaeformis)					
	California	whole	7	871	231
Wakame (Undaria pinnatifada)					
	Tasmania	tablets	4	22	1
		whole	4	41	14
	Japan	whole	6	42	17

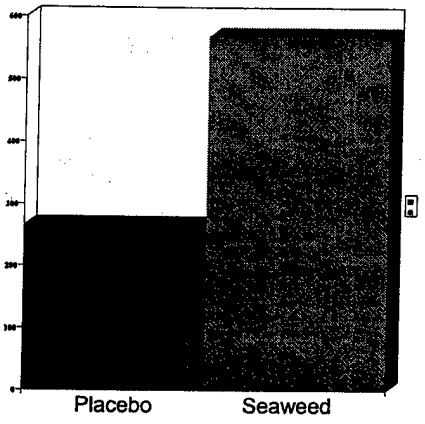
Iodine Within Seaweed

Iodine Content of Individual Seaweed

Form	Stage	Part	N	Mean	± SD
Kelp (Laminaria pallida) (Namibia)					
Sunbleached on shore	adult	meristem			
3 514 (42)					
		blade	3	1034	92
		stipe	3	1452	226
Sunbleached drift in ocean	adult	blade	3	1925	502
			3	1958	251
Fresh	adult	stipe	3	2298	524
		blade	3	3041	702
		meristem	3	4260	1314
	juvenile <50 cm		3	6571	715
Kelp Paddle weed (Ecklonia maxima) (Namibia)					
Fresh	adult	stipe	3	1071	196
			3	1340	441
		blade	3	2500	464
			3	3139	379

Urinary Iodine

24-hr sample



µg/d

P < 0.0001

Conclusions

1. Dietary seaweed was well absorbed by healthy postmenopausal women
2. Serum TSH was significantly increased, within the normal range, during seaweed ingestion.
3. The ingestion of 500 µg seaweed iodine daily resulted in a 300 µg/d increase in urinary iodine excretion.
4. Iodine content of seaweed varies enormously, from 16 µg/g in nori to over 6000 µg/g in kelp
5. Processing of seaweed into powder or granules seemed to increase iodine content, with one sample of seaweed granules having over 8000 µg/g iodine.
6. Iodine content within a single seaweed varied by about 3-fold, with the highest amount of iodine being found in young plants, and blade, especially the meristematic tissue located at the base of the blade.
7. Iodine was lower in sundried seaweed on the beach than in sunbleached drifting seaweed. Iodine content was highest in growing seaweed.
8. Seaweed iodine ingestion could cause thyroid dysfunction in people with underlying thyroid disorders.

Variability of Iodine Content in Common Commercially Available Edible Seaweeds

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Abstract

Dietary seaweeds, common in Asia and in Asian restaurants, have become established as part of popular international cuisine. To better understand the possibility for iodine-induced thyroid dysfunction, we collected samples of the most common dietary seaweeds available from commercial sources in the US, as well as harvester-provided samples from Canada, Tasmania, and Namibia. Altogether, 12 different species of seaweeds were analyzed for iodine content, and found to range from 16 μg/g (± 2) in nori (*Porphyra tenera*) to over 8,165 ± 373 μg/g in one sample of processed kelp granules (a salt substitute) made from *Laminaria digitata*. We explored variation in pre-harvest conditions in a small study of two Namibian kelps (*Laminaria pallida* and *Ecklonia maxima*), and found that iodine content was lowest in sun-bleached blades (514 ± 42 μg/g), and highest amount in freshly cut juvenile blades (6571 ± 715 μg/g). Iodine is water- soluble in cooking and may vaporize in humid storage conditions, making average iodine content of prepared foods difficult to estimate. It is possible some Asian seaweed dishes may exceed the Tolerable Upper Iodine Intake Level of 1,100 μg/day.

Introduction

Iodine was first identified as an element based on the observations of Courtois in 1811 that sulfuric acid-treated seaweed ash produced a purple vapor which condensed into purple crystals (1). For many decades, seaweeds were the primary source of iodine for medicinal purposes, only being supplanted by the discovery that iodine could be extracted from silver and saltpeter mining deposits. These mineral sources were less expensive than collecting seaweed and burning it, and by the late 1930s, seaweed was no longer used as an iodine source (2). In the US, seaweeds have also been used to enrich soil, as sources of inexpensive minerals for animal food supplements, and recently, as food and a natural source of iodine and other minerals in health supplements.

In an earlier study, we and others reported that dietary seaweed delayed the time to tumor onset in a rat dimethylbenzanthracene mammary induced cancer model (3-7). In preparation for a clinical study in humans, it was necessary to evaluate common edible seaweeds for iodine content since excess iodine intake might adversely affect thyroid function. We present our findings for 12 different edible seaweeds, and evaluate factors that modulate seaweed iodine content.

Seaweeds have been a part of the world's ecosphere for two billion years (8), during which time they have changed little. Although archaeological evidence for the use of seaweeds is hampered by its easily degradable nature, it is likely that seaweeds have been part of traditional diets of coastal dwelling peoples worldwide. Seaweeds have been used

by humans as medicine and food for at least 13,000 years,. Based on discoveries of seaweeds found at Monte Verde, a late Pleistocene settlement in Chile (9,10) and archeological inference from ancient sites of the Jomon period in Japan (11), seaweeds have been included in three of the major medical traditions: Ayurvedic medicine from the 4th century BC (12); Traditional Chinese Medicine, with its first reference to its medicinal qualities reportedly made by Shen-nung, in the Chinese "Materia Medica of 2700 BC (13); and in the Ebers Papyrus, the Egyptian treatise on medical care, written approximately in 1550 BC(14). The medicinal uses of seaweed are vast and range from topical burn therapy to goiter therapy to softening of tumors (15). As food, seaweeds have been treasured by the nobility of Japan and China, as well as commoners such as fishermen living along the coast. In 600 BC, Sze Teu wrote, "Some algae are a delicacy fit for the most honored guests, even for the King himself(11)." Tax records from the 8th century indicate that over 30 kinds of seaweed were listed as tax payments to the Japanese government(11). National Seaweed Day (February 6) in Japan commemorates this official recognition of the value of seaweed (16), and the continuing high status of seaweed in Japanese culture. In a recent article on improving public health nutrition in Japan, a recommendation for increasing seaweed consumption was included (17).

The actual amount of seaweed consumed is difficult to quantify as it is often as flavoring to noodles, soups, garnishes, and added as part of mixed vegetable dishes, as well as being a food that is served as a distinct entity as a snack, salad or side dish. In addition, seaweed is a part of military and religious ceremonial celebration foods in Japan. Soups containing seaweed (miso or wakame) have traditionally been included as part of most

meals in Japan (8), although this is changing towards more western foods (18). Estimated iodine intake of people in Japan, mostly from seaweed, ranges from 200 to 20,000 μg/d, with the average estimate of 500-1,000 μg/d) (19). The average seaweed intake in Japan is approximately 4-7 g/d (11,20,21). Commercial data on seaweed sales in Japan estimate that the national average seaweed consumption per person is 4 kg/yr, or closer to 10 g/person/d {Fisheries Information Newsletter 95 #52, 2000 #52}.

In Japan, 21 species of seaweed are routinely included in the diet and in Korea more than 40 kinds of seaweed are commonly used as food (11). Elsewhere in the Pacific basin, in Hawaii and other Polynesian islands, 29 kinds of seaweed have been reported as food, medicine, and as part of religious celebrations in pre-colonial times (23, 24), and seaweeds are still part of the diets of many indigenous people living in Asia, Polynesia and the Pacific Islands.

Seaweeds are increasingly common foods and food supplements in the US. As international foods have become commonplace throughout the world, seaweed consumption has increased. One study of marketing trends reported that 15% of Americans enjoy Japanese cooking (25). Popular claims for seaweed are that they provide an all-natural source of minerals. Such claims of efficacy have placed seaweeds in the role of nutriceuticals as well as regular food.

Methods and Materials

We obtained seaweed samples from health food stores in central Massachusetts, by contacting seaweed harvesters in Tasmania, Maine, and British Columbia, and collecting samples of kelp (*Laminaria* and *Ecklonia)* of known age and condition from Namibia. All samples were in the form that an average consumer might buy.

Samples were analyzed according to the standard determination of total iodine as outlined by Benotti, et. al.(26). This uses the reduction-oxidation reaction between ceric and arsenite catalyzed by iodide. The iodine concentration is proportional to its catalytic activity. Twenty-five-50 mg samples were digested with chloric acid and diluted with iodine free deionized water. They were then measured spectrometrically at 420 nm with a Technicon Autoanalyzer (Technicon Instrument, Inc, Tarytown, NY). Values in the seaweed were based upon an iodine standard curve. At least three aliquots from each seaweed were analyzed for iodine content. The mean value is presented ± standard deviation (SD).

Results

Iodine content of seaweeds varied from 16 μg/g to 8165 μg/g (Table 1). The highest iodine-containing sample came from kelp granules that had been made from *Laminaria digitata* harvested off the coast of Iceland. The kelp granules were made of dried and pulverized seaweed.

In Figure 1 we compare the common edible seaweeds by iodine content. It is interesting to note that American wakame (*Alaria*) and kelp (*Laminaria*), both remarkably similar in appearance, have a marked difference in iodine content, even when both specimens were harvested on the same day by the same harvester from the same bay.

Iodine has been reported to vary with age and condition of the plant, with iodine loss thought to occur rapidly once a seaweed is no longer growing. In Figure 2, sun-bleached seaweed collected from the beach had the lowest iodine content, followed by samples collected from floating drifts of seaweed. Samples cut from growing juvenile (< 50 cm), had approximately twice the amount of iodine/g as found in samples from adult seaweed.

Figure 3 presents the iodine content in different parts of a single seaweed sample. The iodine content in the inedible stipe was approximately twice as high as the blade. Meristematic tissue, the growing area at the base of the blade of the seaweed, was available for *Laminaria* from Namibia. The higher concentration of iodine in this area of the seaweed supports the idea that iodine is important to rapidly dividing seaweed cells.

Discussion

Dietary seaweeds have great variability in iodine content. The problem is further compounded by the use of some high iodine seaweeds as flavoring in some traditional Asian soup stocks and other dishes, in which the seaweed is removed before serving. A

range of post harvest factors affects iodine content of seaweed, including preparation and storage conditions.

The iodine in common edible seaweeds is mostly water soluble, with highest levels reported in kelp (*Laminaria*), of which 99.2% is water soluble (27), although another edible seaweed, *Sargassum*, had only 40% water soluble iodine. The bioavailability of the seaweed iodine to humans has been reported (28-30). Hou reported that the chemical species of iodine in common seaweeds were primarily I^- (66% (*Sargassum*) to 88% in kelp (*Laminaria*)(27). Organic iodine ranged from 10% in kelp to 29% in *Sargassum*, and iodate (IO_3^-) from 1.4% in kelp to 4.5% in *Sargassum (27)*.

Geographic variation in iodine content is also a factor, and iodine values for seaweeds from our study and other published work is presented in Table 2. For example, iodine in wakame (*Undaria*) was 23 times higher in the sample from China, and 2.5 times higher in the samples analyzed by Lee (31) than in the samples we analyzed from New Zealand, Australia, and Japan. Kelp varied from an average of 1542 μg/g in the 10 species of *Laminaria* in our study to 5307μg/g in the values reported for French *Laminaria* (30). The red seaweeds (*Rhodophyta*) analyzed (dulse and nori) were consistently lower in iodine, less than 100 μg/g.

In studies of iodine loss due to storage conditions, Marchal reported that iodine content remained more or less constant when stored in watertight bags or boxes, but lost almost half of its iodine content in the first 40 days when stored in open containers or in paper

bags, especially under humid conditions (29), in which moisture might condense to concentrate water soluble iodine from the seaweed.

Additional factors known to affect iodine content of seaweed are season, salinity of the water, and depth of the seaweed, coldness of the water, distance from the equator, post harvest storage conditions, and possibly other factors (32).

Other factors that affect the iodine content of seaweed include the part of the seaweed used. The stipe (stalk), although generally regarded as inedible, could be included in specimens harvested for health food supplements. In our study, the stipe (base) of the seaweed and the meristematic tissue at the base of the blade had the highest iodine content. Harvesting regulations in places such as Maine require leaving the base and at least 16 inches of seaweed on the rock, which would protect consumers from the high iodine containing stipe and meristematic tissues. However, these guidelines may not be followed by harvesters internationally (33).

Food preparation and cooking methods are other factors in determining final iodine content of foods. Iodine in seaweed is highly water-soluble. One study of the effects of cooking on kelp reported that following 15 minutes of boiling, 99% of the seaweed iodine could be found in the cooking water (34). Although not specifically related to seaweed iodine, cooking loss of iodine from iodized salt has been studied. Goindi (35) reported that the method of cooking was important in iodine loss, with losses ranging from 6% when roasted, to 20% when steamed or deep fried, to 27% when shallow fried,

to 37% -82% with boiling (36). Although the previous values are for iodine in other foods, it is likely that cooked seaweed would be subject to similar rates of loss.

A meal is never made from only seaweed. Seaweed, when eaten in a meal with other foods with goiterogenic potential, such as cassava, *Brassica* (broccoli, cabbage, cauliflower, bok choy, etc.) and soy, may mitigate the effect of the high iodine in the seaweed.

The activity level of the person eating the seaweed has also been reported to be significant in terms of assessing exposure to seaweed iodine (37). In a study of Japanese male university students, iodine losses in sweat during athletic training were high, suggesting that exercise in hot humid climates could increase iodine daily requirements.

Although exposure to high concentrations of iodine will transiently decrease thyroid hormone synthesis for about 24 hours (acute Wolff-Chaikoff effect (38), continued exposure to excess iodine results in a decrease in the iodide concentrating ability of the thyroid by decreasing the thyroid sodium/iodide symporter (NIS), permitting normal thyroid hormone synthesis to resume (38,39). However, some individuals do not escape or adapt to the transient decrease in iodine-induced thyroid hormone synthesis, i.e. those with autoimmune thyroid disease (Hashimoto's thyroiditis), and continued excess iodine ingestion may induce hypothyroiditis (40). Thus, excess ingestion of seaweeds could, in susceptible subjects, induce hypothyroidism and, far less commonly in the United States, hyperthyroidism. Episodic dietary exposure to high iodine-containing foods could pose

health risks for iodine sensitive patrons. For example, the iodine content of nori, the flat sheets of seaweed used to make sushi, contain trivial amounts of iodine (16 μg/g), but a bowl of miso soup made with a kelp flavored stock, even without the presence of seaweed in the final soup, could contain more than 1000 μg of iodine. These sources of natural variation in iodine content contribute to the confusion in describing a particular species as "safe" for iodine sensitive individuals.

Seaweed iodine presents an interesting study in how people in Japan and Korea, with habitual exposure to seaweed-containing diets could be relatively immune to any effects of high iodine intake, but a single seaweed rich Japanese meal could present health risks to iodine sensitive unhabituated diners in low-iodine consuming countries. A greater awareness of the variability of iodine content of seaweeds will help in defining high-risk foods for sensitive individuals.

The observation of Paracelsus (1493-1541), the founder of toxicology, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy" (41), may need to be amended for iodine in seaweed. The safe dose of seaweed may depend on the kind of seaweed, but also the storage conditions, cooking methods, the climate where the person resides, the amount of physical exercise a person does, the presence of goitrogenic foods eaten with the meal, and the frequency of seaweed consumption.

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Table 1. Iodine Content of Commercially Available Edible Seaweeds

Genus & Species	Common Name*	Harvest Location	Form	N	Total Iodine (µg/g)	Std. Dev.
Alaria esculenta	American wakame	Maine	whole	7	**110**	30
		Maine	whole	5	**431**	104
Ascophyllum nodosum	Knotted wrack	Maine	whole	3	**646**	392
Ecklonia maxima	Paddle weed	Namibia	whole	6	**2123**	352
Eisenia bicyclis	Arame	Japan	whole	3	**586**	56
Fucus vesiculosis	Bladderwrack	Maine	whole	3	**276**	82
Hizikia fusiforme	Hijiki	Japan	whole	6	**629**	153
Laminaria	Kelp					
	Oarweed (L. longicruis)	Maine	whole	3	**746**	26
	Kelp**	B.C.Canada	capsule	5	**1259**	200
	Kombu**	Washington	whole	7	**1350**	362
	Wild kelp**	Maine	whole	7	**1356**	665
	Kelp**	B.C.Canada	capsule	5	**1513**	117
	Oarweed	Maine	whole	6	**1862**	520
	Fingered tangle (L. digitata)	Maine	whole	6	**1997**	563
	Mitsuishi-kombu L. angustata)	Japan	powdered	4	**2353**	65
	Fingered tangle	Maine	whole	6	**2984**	910
	Fingered tangle	Iceland	granules	6	**8165**	373
Palmaria palmata	Dulse	Maine	whole	3	**72**	23
Porphyra tenera	Nori, purple laver	Japan	sheet	3	**16**	2
Postelsia palmaeformis	Sea palm	California	whole	7	**871**	231
Sargassum	Horsetail tangle,	WA	whole	5	**30**	1

Undaria pinnatifida	Mekabu (Undaria spore)	Tasmania	tablets	4	**22**	1
	Wakame	Tasmania	powder	5	**32**	4
	Wakame	Tasmania	whole	4	**41**	14
	Wakame	Japan	whole	6	**42**	17
	Mekabu	Tasmania	powder	5	**53**	3
	Wakame	New Zealand	whole	6	**115**	42

* Common names according to Madlener(42)and Arasaki (11)
** No species indicated

Table 2 Comparison of Seaweed Iodine by Genus, Geographic Location, and Study

	This study µg/g	Lee (31) µg/g	Hou (43) µg/g	Aquaron (30) µg/g	Van Netten(44) µg/g
Seaweed origin	US, Canada, Namibia, Tasmania, Japan	UK	China	France	British Columbia
Arame	586	714*			600
Dulse	72	44			
Hijiki	629	391			436
Kelp granules. tablets (salt substitute)	8165	67			815
Kelp/kombu	1542 **	2650	3040	5307	2110
Nori	16	43	36		17 185
Wakame	66***	161*	1571		60 102
Alaria					151

* Average of two reported values
** Average of ten kinds of kelp analyzed
*** Average of three sample sites

Figure 1. Iodine Content of Dietary Seaweeds compared by Species

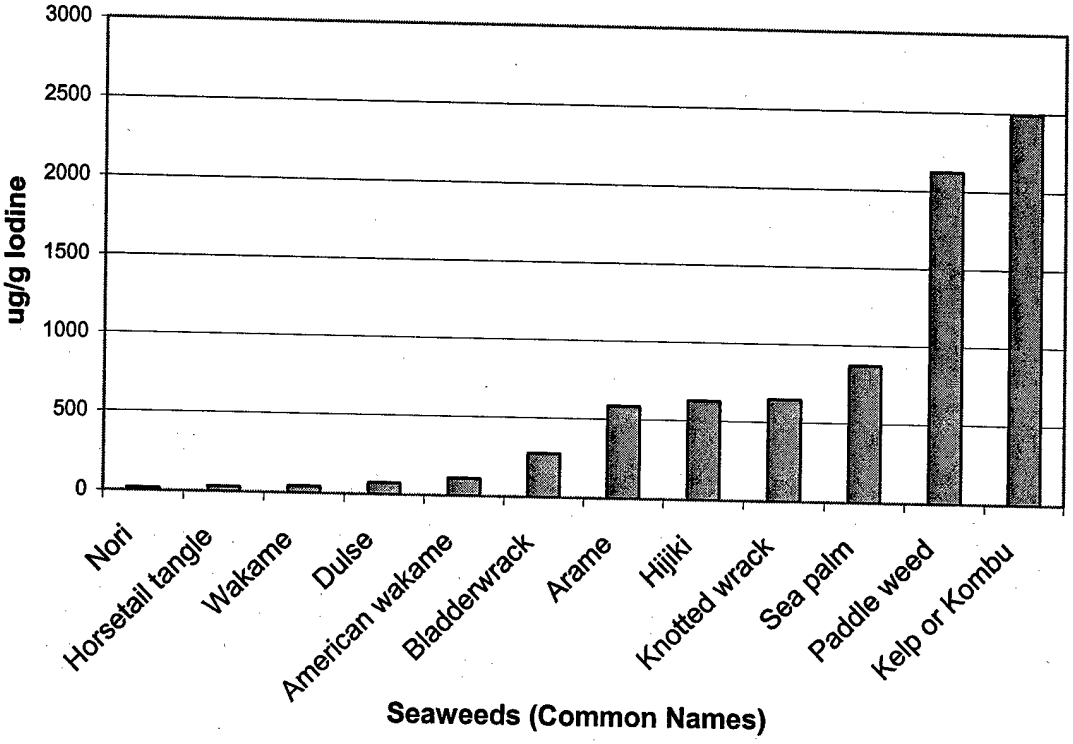


Figure 2. Iodine Content of *Laminaria pallida* Compared by Harvesting Condition and Age of Seaweed Sample

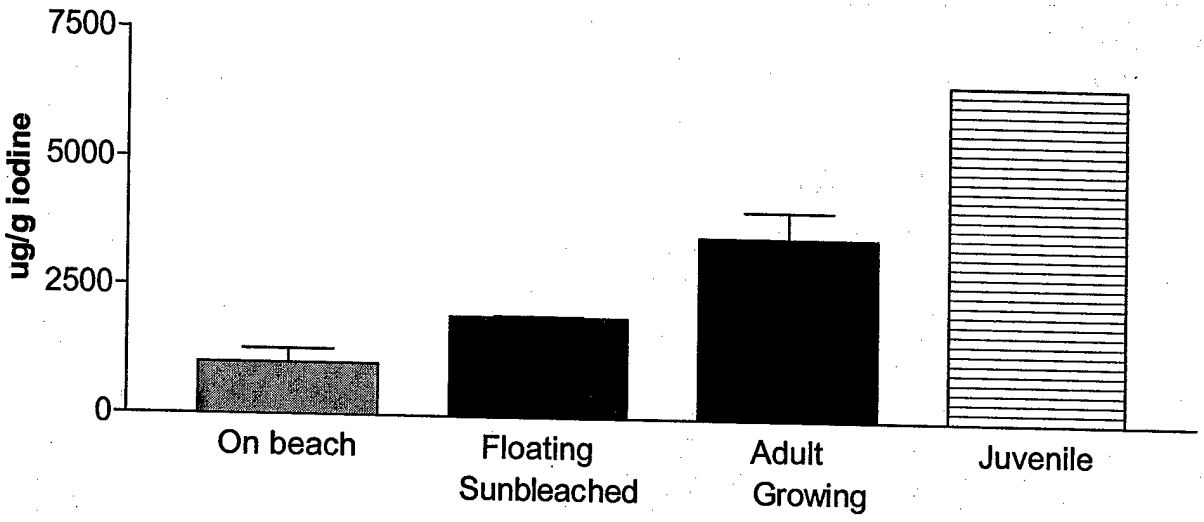
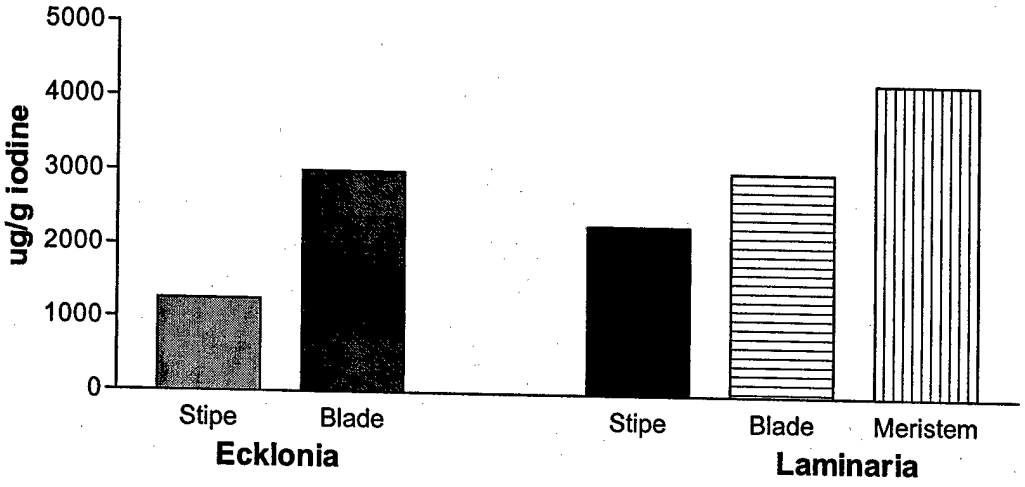


Figure 3. Iodine Content Compared by Part of Adult Fresh Seaweed



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Seaweed and Soy: Companion Foods in Asian Cuisine and Their Effects on Thyroid Function in American Women

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Running title: Dietary Seaweed, Soy and Thyroid Function

Abstract

Seaweeds and soy are two commonly eaten foods in Asia. Both have been reported to affect thyroid function, seaweed because of it iodine content, and soy because of its goitrogenic effect. We were interested in potential toxicities associated with consumption of these foods prior to using them in a breast cancer prevention trial in the US.

Methods: 25 healthy postmenopausal women (mean age 58 yr), 10 of whom had been treated for early breast cancer and 15 who had never been diagnosed with breast cancer, completed a double-blinded randomized crossover study. Ten capsules (5 g/d) of placebo or seaweed (*Alaria esculenta*), providing 475 μg iodine (I)/day, were consumed daily for seven weeks. This was similar to the daily intake of seaweed in Japan. A powdered soy protein isolate (Solae Company) providing 2 mg isoflavone/kg body weight, was given daily during the last week of each treatment arm. On average this provided 141.3 mg/d isoflavones/d and 67.5 g of protein/d. Blood samples and 48-hour urine samples were collected before and after each intervention period, and urinary I/C (μg iodine/g creatinine) and serum T4, FTI, TT3, and thyroid stimulating hormone (TSH) were measured.

Results: I/C concentrations increased significantly ($p<0.0001$) with seaweed ingestion, as did serum TSH ($p < 0.0001$) (1.69 ± 0.22 vs 2.19 ± 0.22 μU/ml, mean ± SE). Serum T4, FTI, and TT3 concentrations did not change significantly. Neither soy protein isolate supplementation nor prior treatment for breast cancer affected thyroid endpoints.

Conclusion: Seven weeks of 5 g/d seaweed was associated with a small but statistically significant increase in TSH. Soy protein isolate supplementation was not associated with changes in circulating thyroid hormone concentrations.

Introduction

Seaweeds are part of many indigenous cuisines around the world, and have been incorporated into some healing therapies, including Traditional Chinese Medicine, Ayurveda, and modern macrobiotics, as well as many folk medicines. On a population level, those people for whom seaweed is a regular part of their diet, most notably in Japan, have dramatically lower breast cancer and prostate cancer rates (1-3). Epidemiologic studies done in the 1980s, before Westernized diets were common, found that Japanese women had 1/3 the rate of premenopausal breast cancer and 1/9 the rate of postmenopausal breast cancer (4). In addition, when a Japanese woman developed breast cancer, she was more likely to survive at least five years longer than women with breast cancer in the United States (5). The histologic type of breast cancer also varies by country, with Japanese women having greater humoral immune responses to the tumors as suggested by the greater degree of lymphocytic invasion of their breast tumors (5).

No previous intervention studies have combined seaweed and soy, but epidemiologic evidence suggests that this combination could be anticarcinogenic. Consumption of miso soup has been associated with reduced breast cancer rates (6-8). Miso soup is usually a combination of soy (miso, tofu cubes) and seaweed (as a stock flavoring and as garnish), and sometimes vegetables.

Seaweed is not necessarily a safe food to consume. We have reported elsewhere that commonly consumed seaweeds have a wide range of iodine concentrations; i.e., between 16 μg/g and 8,000 μg/g (9) making iodine an important dose-limiting factor in seaweed consumption, particularly for people not accustomed to eating seaweed (10). The Lowest-Observed-Adverse-Effect Level (LOAEL), based on increases in serum TSH

in thyroid function challenge tests, is 1,700 μg/d (US and Canadian RDI Committee)(11). However, habituation to high iodine-containing seaweeds appears to be common in Asia, particularly Japan, Korea, and coastal China, where seaweeds are frequently eaten and appear to be well tolerated by millions of people. The average seaweed intake in Japan as approximately 4-7 g/d (12-14), with some estimates as high as 10 g/person/d (15). It is difficult to quantify the actual amount of seaweed consumed as it is often added as flavoring to noodles, soups, garnishes, and may be served as a snack, salad or side dish. Based on dietary intake surveys, the average iodine daily intake is between 500-1,000 μg/d (range from 200 to 20,000 μg/d), with most of the dietary iodine coming from seaweed consumption (16). For this study, we chose a low-iodine containing seaweed (*Alaria esculenta*) to approximate the Japanese average seaweed intake, rather than the approximate iodine intake.

Soyfoods have been suggested as possible human goitrogens (17). The dose of soy protein in our study (average 67.5 g/d) was based on early estimates of average total soy intake in Japan (18). More recent dietary studies indicated that the soy protein intake in Japan is actually lower (10g/d)

The present study determined whether iodine in seaweed was bioavailable and would affect thyroid function, whether a short term soy protein isolate supplementation would depress thyroid function, and whether consumption of these two foods together would have any discernable effect on thyroid function that might be clinically important. Individual contrasts were created to test 3 hypotheses: 1) was seaweed different from placebo (seaweed main effect), 2) was soy different from placebo (soy main effect), and 3) was there an effect from combining seaweed and soy that was different from the

additive effects of seaweed plus soy (seaweed soy interaction). Since the greatest difference between US and Japanese breast cancer rates begins at about age 45 years (20), we studied only postmenopausal women.

Methods

Study population

The University of Massachusetts Medical School Institutional Review Board approved the study. Consent forms were reviewed verbally and all participants gave written informed consent.

Participants were a group of Caucasian American women living in central Massachusetts. We specifically recruited healthy postmenopausal women who had been treated for early breast cancer and women who had never had breast cancer. Women were recruited by word of mouth, by physicians, and through responses to an article in the newspaper. Our inclusion criteria included being postmenopausal (no bleeding for at least one year), intact ovaries at the time of menopause, no history of cancer (other than early breast cancer), no thyroid dysfunction or treatment within the previous 5 years, negative thyroid TPO antibodies excluding Hashimoto's thyroiditis, no hormone replacement therapy within the previous 3 years, no ulcer medications or lithium-based medications, no gastrointestinal disorders such as Crohn's Disease or irritable bowel syndrome, no allergies to seaweed, soy, shellfish or iodine, no treatment with oral antibiotics, iodine-containing medications or corticosteroids within the previous 3 months, no diabetes or high blood pressure medications. In addition, only non-vegetarian women who consumed soy products fewer than two times/week were eligible. Women agreed to avoid eating soy foods during the study, including soybeans and soy products,

as well as sprouts, beans, peas, and lentils, and to restrict alcoholic intake to 1 or fewer drinks/week. Although vitamin and supplement use was allowed, women were asked to refrain from changing dosage or usage during the study. Use of black cohosh, Dong Quai herbal supplements or yam cream were additional reasons for ineligibility.

Forty-eight postmenopausal women were recruited. Based on screening blood samples, we excluded 15 additional women, two for abnormal TSH serum values (either < 0.4 or > 4.5 μU/ml TSH), three for high thyroid peroxidase antibodies (> 191 U/ml), three for current or recent thyroid medications, and seven for lack of interest. Thus, 33 women were enrolled in the study and provided baseline data. Subsequently, four women dropped out during the course of the study due to: a lack of interest (1), naturopath advice (1), allergic reaction (2). Of the two who developed allergic reactions to seaweed, one had red itchy eyes and the other experienced re-activated esophageal reflux. Both conditions resolved spontaneously following cessation of seaweed intake. At the end of the study, two women were excluded because one woman began menstruating again, while the other failed to follow study protocol.

Following publication of studies during the trial on the effects of tamoxifen/roloxifen on thyroid function (21-23), we excluded the three women who used tamoxifen during the entire study, and the one woman who started taking tamoxifen late in the trial had her last 3 observations omitted. Her earlier measures were included in the analysis. This left a final study sample of 25 women among whom 10 had a history of early (Stage I, or II) breast cancer but were disease free at the time of the study and 15 women who had never been diagnosed with breast cancer.

Study Design

The study utilized a randomized, placebo controlled crossover design. Women were randomized to either six weeks of 5-g/d seaweed powder (10 capsules) each evening with the last meal of the day, or six weeks of 5-g/d maltodextrose in 10 identical gelatin capsules. For one additional week, women received either seaweed capsules or placebo capsules and the high isoflavone phytoestrogen powder. To minimize possible effects of season, all women began the study the same week in late October. Samples were collected a total of 7 times (blood samples and 48-hr urine collection) throughout the study. A three-week washout period separated the two arms of the study, and a final three-week washout period followed the end of the last supplements (Figure 1).

Randomization was done using a computer generated random number table. In addition, to assure blinded laboratory analysis, each patient at each clinic visit was assigned a unique ID number.

Seaweed:

We used *Alaria esculenta*, also known as American wakame, a low-iodine (95 µg/g) containing seaweed. The *Alaria esculenta* was harvested at an extremely low tide from the subtidal rocks of the Sally Islands, located near Stuben, Maine. The blades of *Alaria* were cut by hand, placed in plastic baskets, and transported from the islands to the shore in a separate boat made especially for hauling seaweed which was towed behind a larger boat. No gasoline or other potentially toxic substances were present in the seaweed barge. Within 1 hour of harvesting, the seaweed fronds were hung on untreated wooden racks to sundry. After about 10 hours in the sun, the fronds were gathered in bundles, placed in plastic bags, and stored at ambient room temperature in a dark room until shipping, which was done within two months of harvest.

Encapsulation:

The seaweed was shipped by overnight mail to Beehive Botanicals, a subsidiary of Twin Labs (Hayward, Wisconsin), where it was tested for mold and fungus, and found to be negative. The seaweed was ground and encapsulated into gelatin capsules. No fillers or binders were added to the seaweed powder.

Placebo:

Maltrin M100 Maltodextrin (Grain Processing Corporation, Muscatine Iowa) was used for placebo. The daily dose of 5 grams per day provided 18 kilocalories of food energy. We used the same capsules for Maltodextrin and the seaweed. No fillers or binders were added to the placebo powder.

Iodine content of finished capsules

Gelatin capsules were used, and when analyzed for iodine content were found to contain no iodine. The finished seaweed capsules were analyzed, and each capsule contained 47.5 μg of iodine. Thus for our study, we provided an additional 475 μg of iodine/day.

Soy protein

Soy powder (Supro High Protein Nutritious Food Ingredient Powder (with Isoflavones) (Lot #G198-8) was provided by Solae Company (formerly Protein Technologies, Inc. St. Louis, Missouri). It contained 1.43 mg total aglycone (unconjugated or free) isoflavone per gram soy powder. We calculated the appropriate dose for each subject based on her weight, so that each woman consumed 2 mg isoflavones/per kg body weight. On average, each woman was given 67.5 g/d of soy protein. This provided 141.3 mg/d isoflavones, 376 calories, 2.2 g of fat, 22 g of

carbohydrates, and supplied 539 g calcium. Subjects were advised to consume the soy protein isolate as a substitute meal during the two weeks of soy supplementation.

Thyroid hormones:

Chemiluminometric immunoassays (Chiron Diagnostics, East Walpole, MA) were used to measure serum TSH, T3, T4 and T3RU (THBR). The free T4 index (FTI) was calculated as the product of THBR x T4. A chemiluminometric ELISA immunoassay was used to measure anti-TPO-AB (ALPCO, American Laboratory Products Co., Wyndham NH 03087) and was sensitive to 5 IU/mL. All of these assays and urinary creatinine were measured by the Endocrine-Hypertension Laboratory at the Brigham & Women's Hospital, Boston, Massachusetts.

Iodine

Iodine in urine, empty capsules, and finished capsules was analyzed using the ceric-arsenic redox reaction. Samples were analyzed according to standard determination of total iodine protocol as outlined by Benotti, et. al. (24). This used the reduction-oxidation reaction between ceric and arsenite catalyzed by iodide. The iodine concentration was proportional to its catalytic activity. First iodine was precipitated with perchloric acid and the samples were digested with chloric acid. They were then measured spectrometrically at 420 nm with a Technicon Autoanalyzer (Technicon Instrument, Inc., Tarrytown, NY). Calculations were based on an iodine standard curve. The urine results were calculated as iodine/dl, per gram creatinine or total urinary iodine/d.

Urine Collection

Women collected 48-hour urine specimens in 3-liter containers to which 3 grams of ascorbic acid powder had been added. Women stored the collection jugs in their refrigerators until they came in for their next clinic visit (within a day of the end of the collection period). After completely mixing the contents of the jugs, aliquots were taken and stored at -20° C until analysis.

Statistical analyses

Analyses were conducted on 25 women using an intention to treat approach. The study sample characteristics are presented using descriptive statistics. To test the main study hypotheses, a repeated measures analysis of variance was conducted using Proc Mixed in SAS (25). In these models, subject was fit as the repeated factor, while the independent variables treatment group (placebo, seaweed; soy and seaweed plus soy), treatment arm (treatment followed by placebo or placebo followed by treatment) and disease status (history of breast cancer: yes or no) were fit as independent variables. Individual models were run for 7 dependent variables: serum TT3, T4, FTI, TSH and urinary iodine (24 hr excretion, concentration/dL, and iodine standardized per gram creatinine). Results are presented as both average concentrations ($\pm$ SD) and least squares means and the differences are tested using the pdiff option of the Proc Mixed procedure.

Results

The demographic and lifestyle characteristics of the 25 subjects are presented in Tables 1 and 2. The only significant difference between the women who had been treated for breast cancer and those who had never had breast cancer was in family history.

Although we had expected that women with breast cancer would have a stronger family history of breast cancer, that was not the case in our study. Two thirds of the control women had a family history of breast cancer compared to only 20% of the breast cancer patients ($p < 0.04$), although there was no difference between groups in number of first-degree relatives who had been diagnosed with breast cancer.

There were no differences in thyroid function during seaweed and/or soy ingestion in the women who had been treated for early breast cancer but were disease free compared to women who never had breast cancer.

Table 3 presents average serum measurements of thyroid function and urinary iodine excretion in each treatment group. The only significant effects were that seaweed increased urinary iodine excretion and serum TSH (Table 4, Figure 2).

Because the within subject variation in 24 hr urinary creatinine excretion averaged 15% (median 13%), we were concerned that urine collections may have been incomplete for some subjects. Data was analyzed using urinary iodine/gram creatinine concentration, urinary iodine concentration (μg/dL), and by total 24 hour urinary iodine (I/d) excretion. The statistical results did not vary, suggesting that our ability to estimate treatment effects was not effected by this factor. Soy protein isolate supplementation had no significant effect and there was no evidence that there was an interaction between seaweed and soy, when administered together.

BMI of the women varied from 18 to 44. We found no treatment-by-BMI interaction, and although there was a marginally significant association between

weight and TSH, there was no evidence that it influenced our estimation of the seaweed effect.

Discussion

Our data support our first hypothesis that seaweed contains bioavailable iodine, and that consuming seaweed supplements would affect thyroid function. The changes were small and although statistically significant for an increase in serum TSH, the values remained well within normal ranges, and were unlikely to be physiologically important. However, some common dietary seaweeds, especially kelp (*Laminaria*), can contain 40 times as much iodine. The small changes we observed in euthyroid women may not be representative of the effects of high iodine kelp in the general population.

Soy protein isolate supplementation has been associated with goiter formation in iodine deficient rats and humans (17, 26). In our study of iodine-replete subjects, none of the women experienced clinically significant changes in thyroid hormones or urinary excretion of iodine during either of the two one-week periods of high isoflavone soy powder supplementation. We adjusted the dose of isoflavones to body weight (2 mg isoflavones/kg/d), resulting in an average daily intake of 67.5 g soy protein, or about 6-7 times the daily intake of soy protein in Japan (19). Three other studies have investigated the longer term effects of soy protein isolate on thyroid function (27-29). Duncan followed 18 postmenopausal women who used the same dose of soy protein isolate supplement for three months and found no change in thyroid hormones (27). Bruce, who followed 38 postmenopausal women who took a lower dose of soy (30 mg/d isoflavone) for six months, also reported no change in serum thyroid hormones. Only Persky, who studied 46 postmenopausal women who consumed either a similar high (90 mg/d)

isoflavone soy protein isolate (ISP 90, Protein Technologies) or moderate (56 mg/d) isoflavone (ISP56) for six months. reported small but statistically significant changes in thyroid function (TSH was significantly higher at 3 and 6 months for women taking ISP90, significantly higher T4 for women taking ISP56 at both 3 and 6 months; significantly higher T3 at 6 months for women taking ISP90). However, it is difficult to interpret the data as the values of serum thyroid hormones for the women in the three groups (control, ISP56 and ISP90) differed at baseline. All values remained within normal limits (29). Our results from two separate one week exposures to high soy protein isolate provide additional support for the hypothesis that soy supplementation in iodine replete populations have minimal or no effects on thyroid hormones.

The urinary excretion of iodine averaged less than the ingested iodine present in the seaweed. On average, the women in our study excreted 266 μg I per day (13.48 μg/dl during the control period and 587 μg I/d (31.52 μg/dl) while ingesting seaweed. Thus, our subjects were iodine sufficient at the beginning of the study and slightly above average for the United States (30). Exposure to dietary iodine in Asia is much higher. For example, in Japan, the mean urinary iodine excretion in apparently healthy men and women in Sapporo was 5,100 μg/d (n = 4138) (31) and 211μg/dL in Korea (n = 207) (32), compared to a median of approxinmately 217 μg/d in 20,369 Americans(30). When urinary iodine is standardized by creatinine content, the women in our study excreted 291 ug/g when taking placebo, and 587 ug/g when taking seaweed. This is close to 673 μg/g creatinine reported from 278 healthy adults in Korea (33), where seaweed intake is common, and suggests that our study used seaweed exposure that approximates

that found in Asia, where a variety of different kinds of seaweeds are eaten daily in small amounts.

In the only other study of bioavailability of iodine from seaweed, Aquaron reported that in 18 healthy iodine replete volunteers in Marseille, France, 90% of the seaweed iodine was excreted over a 48 hr period, but in a mildly iodine deficient population in Brussels, Belgium, only 62% of the seaweed iodine was excreted in 48 hr (34). In our study in an iodine sufficient population, 60% of the seaweed iodine was excreted in the urine. It is unclear why this was the case. When we compared within subject variation in total urinary creatinine content of the collection specimens, the coefficient of variation averaged 15 ± 7%, with a range between 4% and 30%. We hypothesize that some of the urine collections for some women at a few time points may have been incomplete. It is possible that our estimates of total iodine excretion were biased and that actual iodine excretion was higher than what we recorded.

In Japan and Korea, exposure to high iodine containing foods usually begins *in utero* and via breast milk (35). A study of 50 lactating Korean mothers reported the average maternal iodine intake immediately postpartum was 2744 μg/d, decreasing to 1295 μg/d at 4 weeks. The iodine concentration of the colostrum was 2170 μg/l and breast milk at 4 weeks contained 892 μg/l. This supports the possibility that early exposure to high iodine may be important in the habituation of people living in Asia to high ambient levels of dietary iodine.

Even though we report a small rise in serum TSH with moderate seaweed iodine exposure, thyroid disease in Japan is less common than in the United States. The prevalence of hyperthyroidism (TSH < 0.15 μU/L) among 4110 people living on

Hokkaido in Japan was 0.6% among people living in the capitol of Sapporo, and 1.1% among people living along the coast, and presumably having more seaweed in their diets (31). By comparison, the prevalence of hyperthyroidism (TSH < 0.1 μU/L) among 13,344 adults with no underlying thyroid problems in the US was 1.3 % (36). A slightly more stringent definition of hyperthyroidism in the US (<0.1 μU/L) than in Japan (< 0.15 μU/L) may partially explain the minor differences in rates between the two countries. Hypothyroidism (TSH> 5.0 μU/L) prevalence in Japan was 1.3% in Sapporo and 3.8% along the coast. The US prevalence of hypothyroidism (TSH > 4.5 μU/L) was 4.6%. Again, the slightly different cutoff points may explain the greater prevalence in the US. Age standardized incidence of thyroid cancer rates are higher in the US (6.2/100,000), compared to Japan (4.8/100,000), but the death rates from thyroid cancer are twice as high in Japan (0.6) as in the US (0.3) (20). Using autopsy studies to estimate the rate of undetected thyroid cancer at death, the prevalence in Japan was 35% (37), compared to 3.6% in the US (38). This high prevalence of occult thyroid tumors (35%) but uncommon clinical diagnosis (4.8 ASR) and mortality (0.6 ASR) in Japan suggests that factors such as diet or environment play a role in thyroid cancer initiation and progression. People who immigrate from Japan to the US have higher rates of many cancers, including thyroid cancer (39).

The safety of a high seaweed iodine supplements in the United States, where people have not been exposed since infancy, may be different than it is in Japan. Based on reviews of thyroid status of people in the United States, between 9.5% to 24% of women older than 60 years of age had evidence of thyroid dysfunction (TSH > 5 mIU/L) (36, 40). Even among people known to have hypothyroidism, a recent study found that

40% of people who were taking thyroid medications had abnormal serum TSH levels (40). These baseline characteristics of US populations suggest that low iodine containing seaweeds would be safe, but the more typical high iodine seaweeds (kelp) deserve further study. Since older women have a high prevalence of Hashimoto's thyroiditis which predisposes them to iodine induced hypothyroidism or nodular goiter predisposing them to iodine induced hyperthyroidism, careful monitoring of thyroid function is advisable in iodine exposed women with positive thyroid antibodies or nodular goiter (10, 41).

With any dietary change there is the question of possible harm. In addition to thyroid function, long term exposure to seaweed and thyroid cancer risk need to be considered. Reports of an association between thyroid disease and breast cancer have been found in some, but not all, epidemiologic studies (42). In the largest case control study including 9,257 American women (4575 cases and 4682 controls), the only significant association between thyroid disease and breast cancer was that parous women who had been treated for thyroid cancer had an increased risk of breast cancer (43). Likewise, a large retrospective study of 41,686 breast cancer patients and 3,662 thyroid cancer patients seen at MD Anderson Hospital, reported a significantly increased risk of developing breast cancer after thyroid cancer in young women (44). This finding was explored further using the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database with 1,333,115 person years of data. Women who were diagnosed with thyroid cancer had a significantly increased risk of developing breast cancer. Again, those who had been treated for thyroid cancer had an increased risk of breast cancer (RR 1.18, $p = 0.007$). The effect was most pronounced in premenopausal

women (RR 1.42, p = 0.001) (45). It is possible that therapy of thyroid cancer with large doses of ^{121}I may play a role in this association.

In a more general sense, the role of iodine, as opposed to thyroid function, may be important, as there is some suggestion that iodine deficiency is a risk factor for breast cancer (46-48). Additionally, therapeutic success using oral iodine for breast fibrocystic disease has been reported.(49, 50). Venuri hypothesized that iodine is a primitive antioxidant that has been evolutionarily conserved, and provides protection to cell membranes (46). Iodine is critical for the health of newborn infants, and during lactation and in rapidly dividing breast cancer cells, identification of the mammary gland iodide transporter (sodium iodide symporter, NIS) protein (51), further supports the hypothesis that iodine may be involved in breast cancer. The in vivo data are also suggestive of a role for iodine in mammary tissue protection. Three studies by Funahashi explore the relationship of iodine and iodine in seaweed as factors in inhibition of DMBA-induced mammary tumors in rats (52) (53, 54). As a possible mechanism, Funahashi reported a high correlation between serum iodine and apoptosis of mammary cancer cells. These results, along with those we (55) and others (56-58) have reported for dietary seaweed as inhibitory of DMBA-induced mammary tumors are supportive of the idea that seaweed, possibly via iodine, could be involved in breast cancer prevention.

Conclusion

In an iodine-replete population of healthy postmenopausal women with normal thyroid function, the ingestion of an additional 475 μg I/d was associated with a small but significant increase in serum TSH. Soy protein isolate had no effect on thyroid function or iodine excretion. A history of treatment for early breast cancer (Stage I or II) did not

alter the changes observed in the seaweed-associated increase in TSH or iodine excretion. However, our sample size was small and all women in the present study were screened to rule out underlying thyroid disease. It is unknown how seaweed ingestion might affect women with underlying thyroid disease. Given appropriate medical supervision and prior screening for allergies to iodine and thyroid function, consumption of low iodine seaweed and soy protein isolate is unlikely to cause serious side effects.

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Table 1 Demographic factors for 25 women*

Characteristic	Breast Cancer N	Breast Cancer %	Disease Free N	Disease Free %	p-value
Education					
High school graduate or less	2	20	4	27	0.58†
Some college or associate degree	4	40	3	20	
Bachelors degree or more	4	40	8	53	
Ethnicity					
Caucasian American	10	100	15	100	-
Social status					
Living alone	3	30	2	13	0.36†
Living with some one	7	70	13	87	
Employment					
Full time	7	70	9	60	0.83†
Part time	1	10	1	7	
Not working	2	20	5	33	

	Mean	SD	Mean	SD	
Age (yr)	58.4	6.1	58.1	8.5	0.93§
BMI (kg/m^2)	27.2	6.7	26.2	4.4	0.66§

Table2 Lifestyle and health-related factors for 25 women*

Characteristic	Breast Cancer N	Breast Cancer %	Disease Free N	Disease Free %	p-value
General Health					0.72§
Excellent	2	20	5	33	
Very Good	6	60	9	60	
Good	2	20	1	7	
Exercise					0.12§
Yes	5	50	12	80	
No	5	50	3	20	
Alcohol Use†					0.67§
Yes	4	40	4	27	
No	6	60	11	73	
Multivitamin Use					0.69§
Yes	6	60	7	47	
No	4	40	8	53	
Herbal Supplements Use					1.00§
Yes	4	40	7	47	
No	6	60	8	53	
Hysterectomy					0.27§
Yes	3	30	1	7	
No	7	70	14	93	
Ever pregnant					1.00§
Yes	9	90	13	87	
No	1	10	2	13	
Menopausal symptoms					0.24§
Yes	6	60	5	33	
No	4	40	10	67	
Self medication for menopausal symptoms¶					1.00§
Yes	3	30	5	33	
No	7	70	10	67	
Family history of breast cancer					
Yes	2	20	10	67	0.04§
No	8	80	5	33	

If yes, first degree breast cancer					0.45§
N/A?	8	-	5	-	
Yes	1	50	8	80	
No	1	50	2	20	
If first degree, breast cancer type					1.00§
Pre menopausal	0	0	1	13	
Post menopausal	1	100	7	87	
N/A	8	-	5	-	
Missing	1	-	2	-	
	Mean	**SD**	**Mean**	**SD**	
Age at 1st pregnancy	24.2	3.4	24.3	3.7	0.24++
Age at menopause	48.8	3.5	50.5	2.2	0.15++
Social support **	17.9	9.9	13.1	9.4	0.24++
No. of miscarriages	0.9	1.4	0.4	0.8	0.28++

*This analysis includes 25 women, 10 with a history of breast cancer and 15 with no such history

†1 or fewer drinks per week

§ Fisher's Exact Test

¶ Self-medication of menopausal symptoms with dietary herbal supplements, or over-the-counter drugs

**Social support was defined as the number of friends and relatives a woman was in contact with during a week.

++T test

Table 3. Mean values for thyroid function and urinary iodine excretion by treatment period for 25 women

Thyroid Tests	Placebo		Seaweed		Soy		Seaweed and Soy	
	Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
Serum hormones								
T3 (ng/dL)	**124.1**	16.5	**128.4**	16.2	**125.6**	17.7	**125.8**	16.6
T4 (ug/dL)	**6.56**	0.99	**6.62**	0.90	**6.77**	1.14	**6.68**	0.85
FTI	**5.74**	0.71	**5.75**	0.69	**5.89**	0.94	**5.88**	0.68
TSH (ulU/ml)	**1.69**	0.95	**2.19**	1.23	**1.64**	1.01	**1.94**	1.13
Urinary iodine excretion								
Iodine (ug/d)	**265.8**	155.8	**567.8**	177.8	**290.5**	190.9	**545.6**	136.7
Iodine (ug/g creatinine)	**290.5**	147.8	**586.9**	177.6	**328.5**	185.4	**571.7**	147.8
Iodine (ug/dL)	**13.48**	7.80	**31.52**	12.26	**14.99**	12.16	**28.70**	8.21

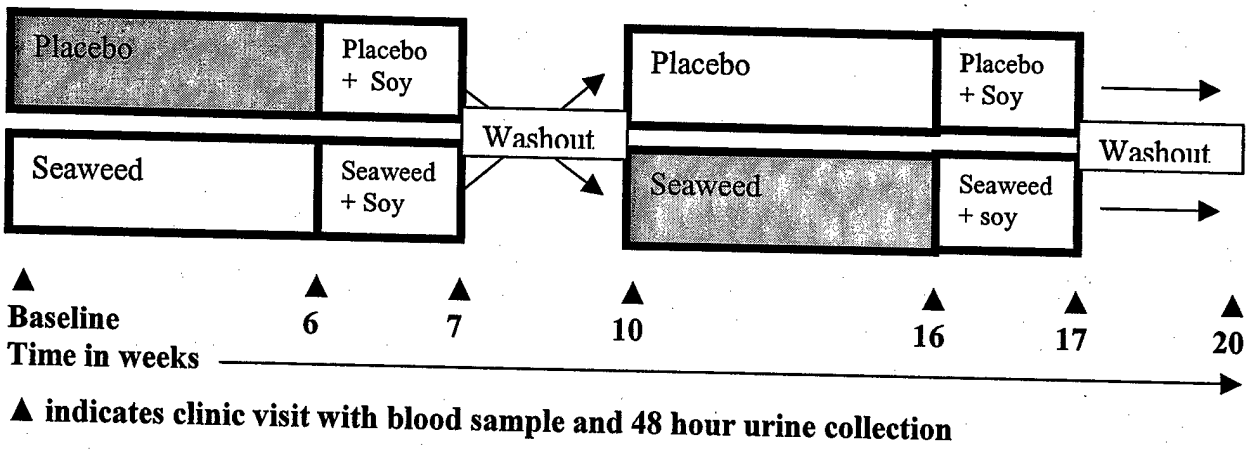
* As this was a crossover study, each woman received each treatment and these values represent the average values in all 25 subjects.

Table 4. Test of the hypothesis that seaweed and soy were different from placebo in their effects on thyroid function

Thyroid Tests	Seaweed*			Soy*		
	Mean	SE	**P-value**	Mean	SE	**P-value**
Serum hormones						
T3 (ng/dL)	4.28	2.32	0.07	0.72	2.35	0.76
T4 (ug/dL)	0.06	0.15	0.71	0.19	0.15	0.20
FTI	0.01	0.13	0.94	0.15	0.13	0.27
TSH (ulU/ml)	0.50	0.10	**0.0001**	-0.04	0.10	0.67
Urinary iodine excretion						
Iodine (ug/d)	302.0	29.7	**0.0001**	24.7	30.1	0.41
Iodine (ug/g creatinine)	296.4	39.1	**0.0001**	38.0	39.6	0.34
Iodine (ug/dL)	18.04	1.74	**0.0001**	1.51	1.77	0.39

* To test for the main effects of seaweed and soy, the values shown are the LSmean difference and its standard error calculated as treatment - placebo. These values were derived from a mixed-model repeat measures analysis of variance by creating a linear contrast in Proc Mixed (SAS). The P-value is for the T-Test of the Ho: the mean difference = 0.

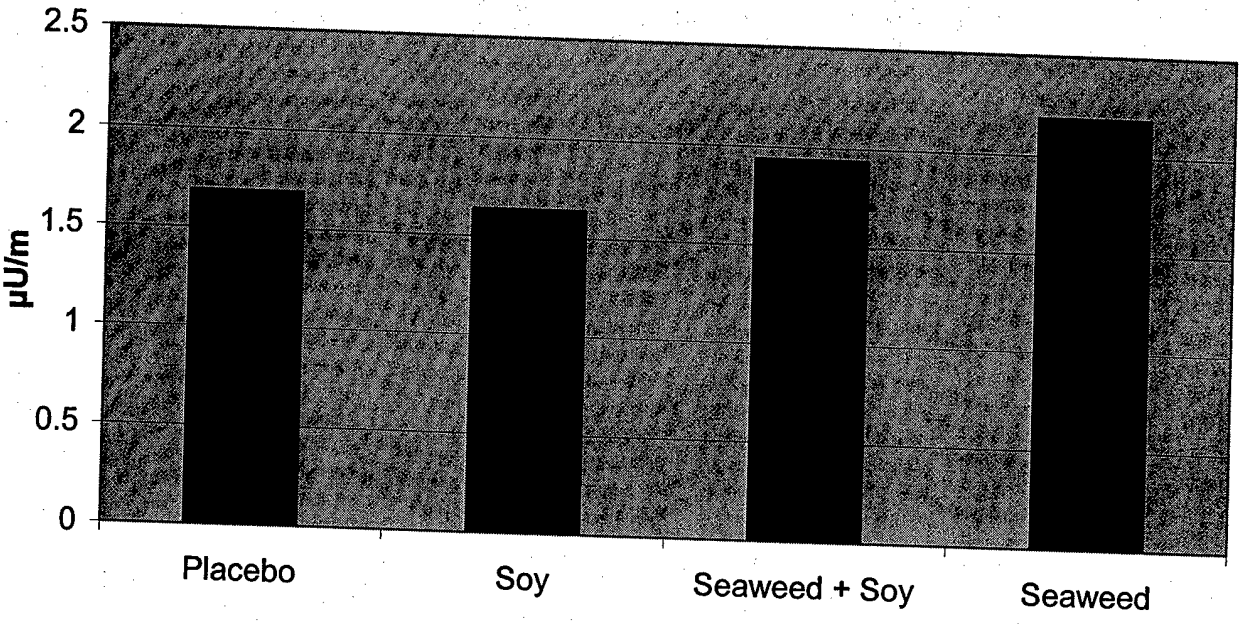
Figure 1. Study Design



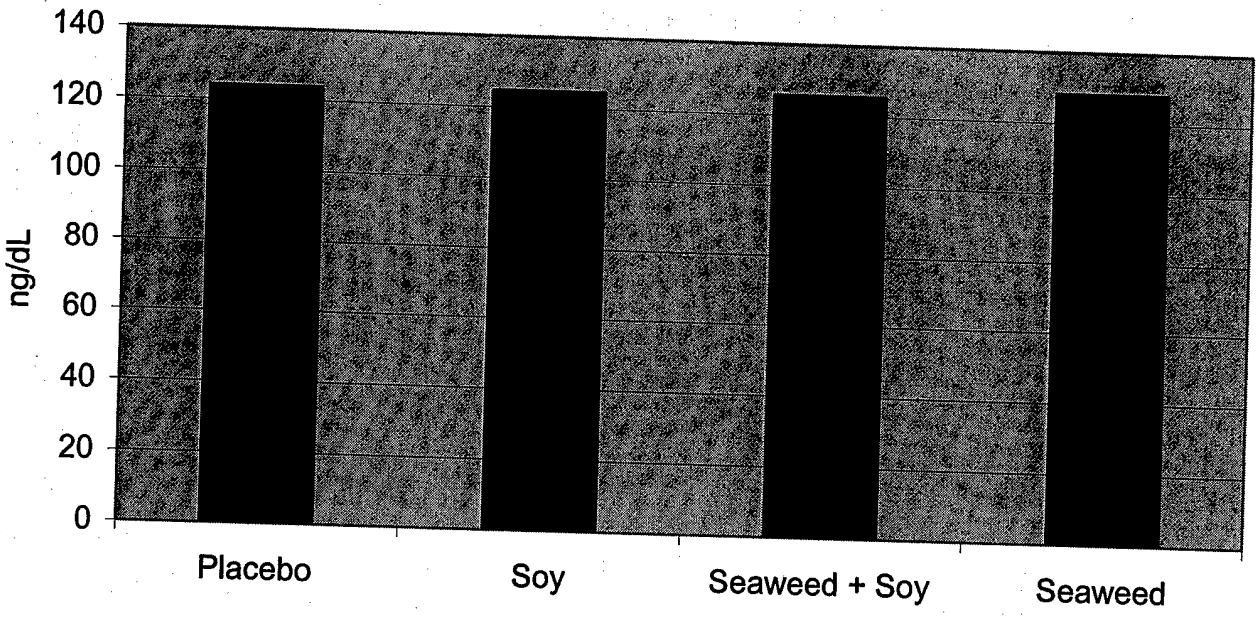
▲ indicates clinic visit with blood sample and 48 hour urine collection

Figure 2. Comparison of Treatment Effects on Thyroid Hormones (TSH, T3, and T4)

TSH



T3



T4